Dual function of Potentilla (Rosaceae) in the life history of the rare boreoalpine osmiine bee Hoplitis (Formicapis) robusta (Hymenoptera, Megachilidae)

Müller, Andreas ; Richter, Henning

Abstract: Hoplitis robusta (Nylander) is a rare and poorly known osmiine bee species occurring in the subalpine zone of the Alps. The discovery of two nests of H. robusta in a thin branch of a dead fallen spruce on a sunny clearing of a subalpine spruce forest allowed the investigation of the nest architecture, the analysis of the larval diet and the assessment of the nest building material. X-raying, computed tomography and subsequent dissection of the nest branch revealed that the nests were built in L-shaped pupation tunnels of cerambycid beetles, which were probably cleaned from wood debris by the female bees with the aid of their large and powerful mandibles after nest site selection. The two nests contained five and six linearly arranged brood cells separated from each other by thin partitions built from masticated green leaves ("leaf pulp"). They were sealed at their opening by a thick plug consisting of several successive layers of leaf pulp constructed immediately behind each other. Microscopical analysis of the larval provisions of eight brood cells and of 41 pollen loads of females from museum and private collections showed that H. robusta exhibits a strong preference for the pollen of Potentilla (Rosaceae). Based on field observations, DNA metabarcoding of one nest plug and stereomicroscopic analysis of the leaf pulp matrix, Potentilla was also identified as an important source for the leaf pulp needed for nest construction, rendering H. robusta one of the few bee species known to collect floral resources and nest building material from the very same plant.

DOI: https://doi.org/10.3897/alpento.2.30158

Alpine Entomology 2 2018, 139–147 | DOI 10.3897/alpento.2.30158

PENSOFT

Alpine Entomology

Dual function of *Potentilla* (Rosaceae) in the life history of the rare boreoalpine osmiine bee *Hoplitis* (*Formicapis*) *robusta* (Hymenoptera, Megachilidae)

Andreas Müller[1], Henning Richter[2]

1 *ETH Zurich, Institute of Agricultural Sciences, Biocommunication and Entomology, Schmelzbergstrasse 9/LFO, 8092 Zurich, Switzerland*
2 *University of Zurich, Vetsuisse Faculty, Clinic for Diagnostic Imaging, Winterthurerstrasse 258c, 8057 Zurich, Switzerland*

http://zoobank.org/FC387A3A-2E8C-4DD1-A25D-07C3EA7B6C25

Corresponding author: *Andreas Müller* (andreas.mueller@usys.ethz.ch)

Received 28 September 2018
Accepted 5 November 2018
Published 20 November 2018

Academic editor:
Jessica Litman

Key Words

Apiformes
beetle burrows
dead wood
Fragaria
Helianthemum
mandibles
pollen analysis
Potentilla erecta

Abstract

Hoplitis robusta (Nylander) is a rare and poorly known osmiine bee species occurring in the subalpine zone of the Alps. The discovery of two nests of *H. robusta* in a thin branch of a dead fallen spruce on a sunny clearing of a subalpine spruce forest allowed the investigation of the nest architecture, the analysis of the larval diet and the assessment of the nest building material. X-raying, computed tomography and subsequent dissection of the nest branch revealed that the nests were built in L-shaped pupation tunnels of cerambycid beetles, which were probably cleaned from wood debris by the female bees with the aid of their large and powerful mandibles after nest site selection. The two nests contained five and six linearly arranged brood cells separated from each other by thin partitions built from masticated green leaves ("leaf pulp"). They were sealed at their opening by a thick plug consisting of several successive layers of leaf pulp constructed immediately behind each other. Microscopical analysis of the larval provisions of eight brood cells and of 41 pollen loads of females from museum and private collections showed that *H. robusta* exhibits a strong preference for the pollen of *Potentilla* (Rosaceae). Based on field observations, DNA metabarcoding of one nest plug and stereomicroscopic analysis of the leaf pulp matrix, *Potentilla* was also identified as an important source for the leaf pulp needed for nest construction, rendering *H. robusta* one of the few bee species known to collect floral resources and nest building material from the very same plant.

Zusammenfassung

Hoplitis robusta (Nylander) ist eine seltene und erst unzureichend erforschte Bienenart aus der Verwandtschaft der Mauerbienen (Osmiini). Sie kommt bei uns ausschliesslich in der subalpinen Stufe der Alpen vor. Die zufällige Entdeckung von zwei Nestern, welche sich in einem dünnen Ast einer umgestürzten toten Fichte auf einer sonnigen Lichtung eines subalpinen Fichtenwaldes befanden, ermöglichte die Analyse der Nestarchitektur, der Larvenvorräte und des Nestbaumaterials. Die Untersuchung des besiedelten Astes durch Röntgen, Computertomographie und anschliessende Präparation ergab, dass beide Nester in Hakengängen von Bockkäfern (Cerambycidae) angelegt worden waren, welche die Weibchen mit Hilfe ihrer ausgesprochen kräftigen Mandibeln wahrscheinlich vorgängig gesäubert hatten. Die zwei Nester enthielten fünf bzw. sechs linienförmig hintereinander angelegte Brutzellen, die durch dünne Wände aus zerkauten Laubblättern («Pflanzenmörtel») voneinander getrennt waren. Die Nester wurden an ihrer Öffnung mit einem dicken Propfen verschlossen, der aus mehreren direkt hintereinander liegenden Wänden

aus Pflanzenmörtel gebaut wurde. Die lichtmikroskopische Analyse der Larvenvorräte in acht Brutzellen sowie der Pollenladungen von 41 Weibchen aus Museums- und Privatsammlungen ergab, dass *H. robusta* den Pollen hauptsächlich auf Fingerkraut (*Potentilla*) sammelt. Mehrere Beobachtungen von Nestbaumaterial sammelnden Weibchen, die genetische Analyse eines Nestverschlusses und die Untersuchung des Nestbaumaterials unter dem Binokular ergaben, dass *Potentilla* auch eine sehr wichtige Pflanzenmörtelquelle ist. Damit gehört *H. robusta* zu den wenigen bisher bekannten Bienenarten, welche sowohl den Pollen als auch das Nestbaumaterial auf den gleichen Pflanzen sammeln.

Introduction

Bees depend on several types of resources for their reproduction (Westrich 1989). They need suitable nesting sites to hide the brood cells, flowers to collect pollen and nectar and specific materials to build the nests. In some highly specialized Palaearctic osmiine bees (Megachilidae), floral resources and nest building material are harvested from the very same plants. In *Haetosmia vechti* flowers of *Heliotropium* (Boraginaceae) serve as the exclusive pollen source, while masticated green leaves of the same plant are used to construct the brood cells (Gotlieb et al. 2014). Similarly, several pollen specialist *Hoplitis* and *Osmia* species of the subgenera *Anthocopa* and *Tergosmia*, respectively, each build their brood cells from petals of their exclusive pollen host, such as *Centaurea* (Asteraceae) in *Hoplitis saundersi, Convolvulus* (Convolvulaceae) in *H. perezi*, *Linum* (Linaceae) in *H. mocsaryi*, Malvaceae in *H. cristatula* and *H. jakovlevi*, or *Hedysarum* (Fabaceae) in *Osmia avosetta* (Ferton 1890, 1892, 1894, 1897, Westrich 1989, Rozen et al. 2010, Müller 2018a, G. Le Goff personal communication). By exploiting the same plant for different types of resources, these bees likely avoid costs incurred by searching for and exploiting alternative plants. In the present contribution, we report on the dual function of *Potentilla* (Rosaceae) as a source for both pollen and nest building material for *Hoplitis robusta* (Nylander), a rare boreoalpine osmiine bee.

Hoplitis robusta is a holarctic megachilid bee species of 6–9 mm length distributed in the boreal zone across Europe, Asia and North America with outposts in mountainous regions of the western USA and the Alps (Müller and Mauss 2016). In the Alps, *H. robusta* occurs from western Switzerland over northern Italy to eastern Austria, where it mainly inhabits open forests and windfalls between 1200 m a.s.l. and the timber line (Schwarz et al. 1996, Ebmer 1997, 2001, Amiet et al. 2004, Kopf 2008, SwissBeeTeam 2018). Among the osmiine bees, *H. robusta* belongs to the subgenus *Formicapis*, which comprises three additional species all restricted to a comparatively small area that ranges from northern Mongolia and China to southeasternmost Russia and Korea, suggesting an eastern Asian origin of the subgenus (Müller and Mauss 2016).

The biology of *Hoplitis robusta* is only fragmentarily known, which is likely due to the species' rare and scattered occurrence throughout the Alpine arc. Based on observations of individuals flying around dead spruces and wooden fences, *H. robusta* was assumed to nest in insect burrows in dead wood (Frey-Gessner 1880, 1908–1912). This assumption was later supported by the discovery of several nests built in drilled borings of artificial trap nest blocks in the Rocky Mountains/USA (Clement and Rust 1975). Still, however, no nests of *H. robusta* in a natural substrate have ever been found, neither in North America nor in Europe. A similar poor knowledge pertains to the host plant choice of *H. robusta*, which was completely unknown until recently, when *Potentilla* was identified as a seemingly important host based on the microscopic analysis of pollen contained in the metasomal scopa of collected females (Müller and Mauss 2016). However, the low number of pollen samples analysed in the cited study renders it difficult to evaluate the true significance of *Potentilla* pollen in the species' larval diet.

Numerous attempts to find nests of *Hoplitis robusta* in the Swiss Alps during the past decade – including the repeated exposure of bundles of hollow bamboo stems that imitated insect burrows in dead wood – failed. In Summer 2018, however, a female of *H. robusta* was by chance observed sealing her nest in a branch of a dead spruce. X-raying of the branch revealed the existence of a second nest of a different female within the same branch. The discovery of these first truly natural nests of *H. robusta* allowed the investigation of the nest architecture, the assessment of the nest building material and the analysis of the larval diet and prompted the analysis of additional female pollen loads, all aiming at getting a more complete picture of the life history of this rare and enigmatic alpine bee species.

Methods

The nests of *Hoplitis robusta* were discovered in the Calfeisen valley near St. Martin/St Gallen (46°55.455N, 9°21.305E) at an elevation of 1520 m a.s.l. on 27.6.2018. The nest branch was sawed off at its base and brought to the Animal Hospital of the University of Zurich, where it was X-rayed using a Bucky Diagnost CS/TH X-Ray (Philips) combined with a Profect CS Mammo-Reader (Fujifilm). In addition, the two nests were examined by computed tomography (CT) using a 16-slice CT Brilliance scanner (Philips). Following the X-ray and CT-examination, which enabled the exact localization of the nests within the branch, the nests were dissected with the

aid of a smoothing plane and a pocket knife to analyse their architecture and the nest contents.

The pollen host spectrum of *Hoplitis robusta* was assessed by microscopic analysis of the scopal pollen contents of 41 females from museum and private collections originating from 27 different localities distributed across the Alps and from three different localities in Finland. The pollen samples from the Alps (n = 15) analysed by Müller and Mauss (2016) were included in the present study and supplemented with numerous new samples (n = 26). In addition, remains of the larval provisions in eight brood cells of two nests were microscopically analysed. The methodology for pollen removal, manufacture of pollen samples, pollen identification and data evaluation follows Müller (2018b). For each brood cell, the proportions of the different pollen types were estimated to the nearest 10%. Pollen of *Fragaria* and *Potentilla* – two closely related genera within the Rosaceae – could not be reliably separated by the method applied in the present study, both being subsumed under the "*Potentilla* type" in palynology (Beug 2004). As *Fragaria vesca* L. – the only *Fragaria* species occurring in the subalpine habitats of *H. robusta* – flowers early in spring when the flight period of *H. robusta* has not yet started, all pollen grains of the *Potentilla* type were invariably assigned to the genus *Potentilla*.

To assess the source of the nest building material, DNA metabarcoding of the two nest plugs consisting of masticated green leaves (= "leaf pulp") was performed by the ISO 9001:2008 certified and ISO 17025:2005 accredited laboratory of Eurofins Medigenomix GmbH (Ebersberg, Germany). DNA of 1g plant material from each nest plug was extracted with the Maxwell 16 FFS nucleic acid extraction kit (Promega) following the manufacturer's manual. From the extracted DNA, barcoding sequences of the nuclear marker ITS2 and the chloroplast marker trnL were PCR amplified using target specific next-generation sequencing primers and analysed by amplicon sequencing on the Illumina MiSeq platform. For unknown reasons, the extraction of amplifiable DNA from the plug of nest 1 failed. The amplified sequences from the plug of nest 2 were sorted into sequence sets according to their similarity, each represented by a master sequence. The master sequences were identified to species or genus level by comparing them with known plant sequences made available by the NCBI database. We refrained from performing metabarcoding of the cell partitions as they were heavily contaminated with pollen of the larval provisions. Instead, the leaf pulp matrix of all cell partitions and the outermost partition of the plug of nest 1, which was still available after the unsuccessful DNA amplification (see above), was stereomicroscopically examined at a magnification of 40× and compared with the leave indument of plant species collected in a radius of 15 m around the nesting site on 16.9.2018.

Data on flower visits and sources of nest building material are based on field observations in the Swiss Alps near Visperterminen/Valais (46°15.00N, 7°55.50E), St. Martin/St Gallen (46°55.50N, 9°21.30E), Curaglia/Grisons (46°40.25N, 8°50.10E), Sedrun/Grisons (46°41.20N, 8°47.50E), Sent/Grisons (46°48.55N, 10°21.10E) and Vals/Grisons (46°36.90N, 9°11.80E) from 2010 to 2018. A "flower visit" is defined as one continuous observation of a single female irrespective of the number of flowers she visited.

Results

Nesting site and nest architecture

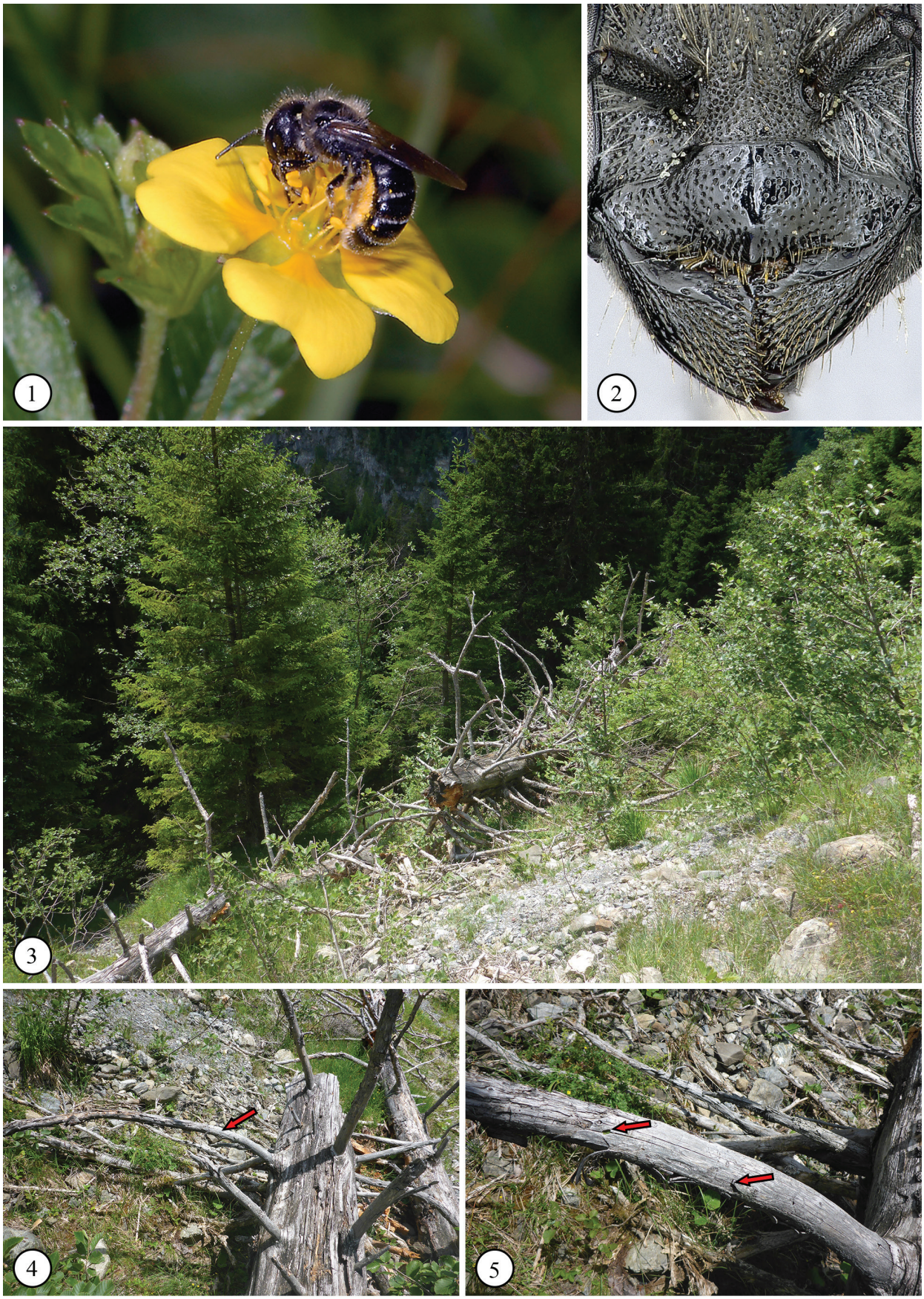
The two nests of *Hoplitis robusta* were found on a sunny clearing of a subalpine spruce forest (Fig. 3). They were situated in a 4 cm thick and 90 cm long branch of a dead fallen spruce (*Picea abies* (L.) H. Karst.) (Figs 4, 5), the nest entrances being in a distance of 31 cm (nest 1) and 20 cm (nest 2) from the branch base. Both nests were built in L-shaped pupation tunnels of an unknown cerambycid beetle species (Figs 6, 9, 10 and Suppl. materials 1, 2). The two L-shaped tunnels had a length of 6.5 cm and 5.7 cm and were oval in shape with a mean diameter of about 6 × 3 mm and 5.5 × 3 mm. Upon discovery of the nests, the female of nest 1 was finalizing the nest seal, whereas nest 2 was already finished. As the oldest bee larva in the innermost brood cell of nest 1 was in a slightly more advanced stage than the youngest larva in the outermost brood cell of nest 2 (Figs 9, 10), the two nests must have been built by two different females.

The two nests contained six and five linearly arranged brood cells all situated in the straight horizontal part of the L-shaped tunnel (Figs 6, 9, 10 and Suppl. materials 1, 2). The brood cells, whose length ranged from 5.5–7.5 mm, were separated from each other by 0.3–0.5 mm thin partitions constructed from leaf pulp without addition of any supplementary material (Fig. 11). Neither nest was sealed by a wall of leaf pulp against its rear end, the larval provision of the innermost brood cell directly contacted the wood at the bottom of the tunnel. In both nests, the outermost brood cell was not closed at its anterior end by a cell partition (Figs 9, 10), the distance between its base and the bottom of the nest plug measured 14 mm and 17 mm.

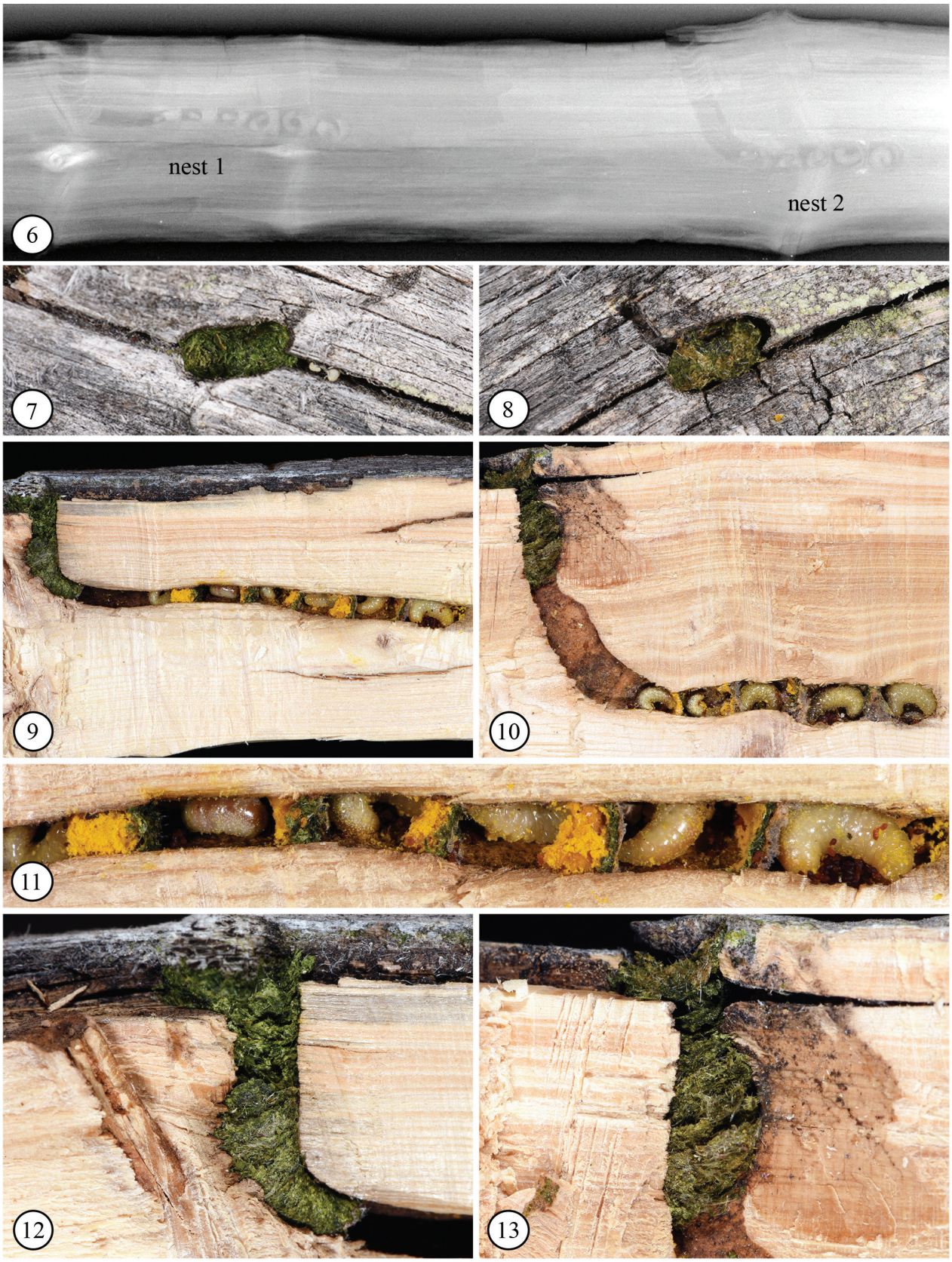
Both nests were sealed by a thick nest plug exclusively constructed from leaf pulp (Figs 12, 13). The two nest plugs had a length of 12 mm and 11 mm and consisted of ten and eight 1–2 mm thick partitions, which were built immediately behind each other (Fig. 6 and Suppl. materials 1, 2) The outermost plug partition consisted of more finely masticated leaves than the inner partitions; its exterior side was smoothed and about 2–3 mm behind the burrow entrance (Figs 7, 8).

Pollen hosts

Hoplitis robusta harvested the pollen of five plant families (Tab. 1). However, it exhibited a strong preference for *Potentilla* (Rosaceae) (Fig. 1), as revealed by the composition of both the female scopal loads and the brood cell provisions (Tab. 1, 2). In the scopal loads, pollen of *Potentilla* constituted 77.1% of the total pollen grain volume and it was recorded in 37 out of 41 loads, 26 of which



Figures 1–5. *Hoplitis robusta*. **1)** Female collecting pollen and nectar on *Potentilla erecta*. **2)** Head of female. **3)** Nesting habitat in the Calfeisen valley near St. Martin (St Gallen). **4)** Dead fallen spruce (*Picea abies*) with nesting branch marked with red arrow. **5)** Nesting branch with nest entrances marked with red arrows.



Figures 6–13. *Hoplitis robusta*. **6)** X-ray of nest branch with nest 1 (left) and nest 2 (right). **7, 8)** Nest seal of nest 1 (left) and nest 2 (right) consisting of leaf pulp. **9, 10)** Dissected nest 1 with six brood cells (left) and nest 2 with five brood cells (right) in L-shaped pupation tunnels of cerambycid beetles. **11)** Close-up view of brood cells in nest 1. **12, 13)** Nest plug of nest 1 (left) and nest 2 (right), each consisting of several successive partitions of leaf pulp.

Table 1. Pollen composition of female pollen loads of *Hoplitis robusta*. n = total number of pollen loads, N = number of pollen loads from different localities. Countries: A = Austria, CH = Switzerland, FIN = Finland. Plant families: AST = Asteraceae, CIS = Cistaceae, FAB = Fabaceae, RAN = Ranunculaceae, ROS = Rosaceae. Definition of bee host range after Müller and Kuhlmann (2008).

n	N	Origin (and number) of pollen loads	% pollen grain volume (number of loads)	Preferred host	% pollen grain volume of preferred host	% pure loads of preferred host	% loads with preferred host	Host range
41	30	A (2), CH (35), FIN (4)	ROS (*Potentilla*) 77.1% (37), ROS (*Rubus*) 1.4% (1), CIS (*Helianthemum*) 13.6% (9), FAB (*Medicago*) 3.2% (1), FAB (*Lotus*) 0.3% (1), AST (Cichorioideae) 1.8% (3), RAN (*Ranunculus*) 1.6% (4), unknown 1% (3)	*Potentilla*	77.1%	63.4%	90.2%	polylectic (5 plant families) with strong preference for *Potentilla* (Rosaceae)

were pure loads. In the cell provisions, pollen of *Potentilla* was the exclusive constituent in six out of eight provisions, while two provisions additionally contained moderate amounts of pollen of *Helianthemum* (Cistaceae). Pollen collection by females of *H. robusta* at six different localites in the Swiss Alps (see Methods) was invariably observed on flowers of *Potentilla erecta* (L.) Raeusch. (n = 27 flower visits), except for two females that visited the flowers of *Potentilla aurea* L. and *Ranunculus lanuginosus* L., respectively.

Pollen collecting females worked the *Potentilla* flowers in a rapid and efficient way (Fig. 1): they embraced the anthers below their body and – while turning slowly around the flower centre – picked up the pollen directly into the scopa by rapidly seesawing the underside of the metasoma against the anthers (Fig. 1); simultaneously, they sucked nectar from the base of the androecium.

Nest building material

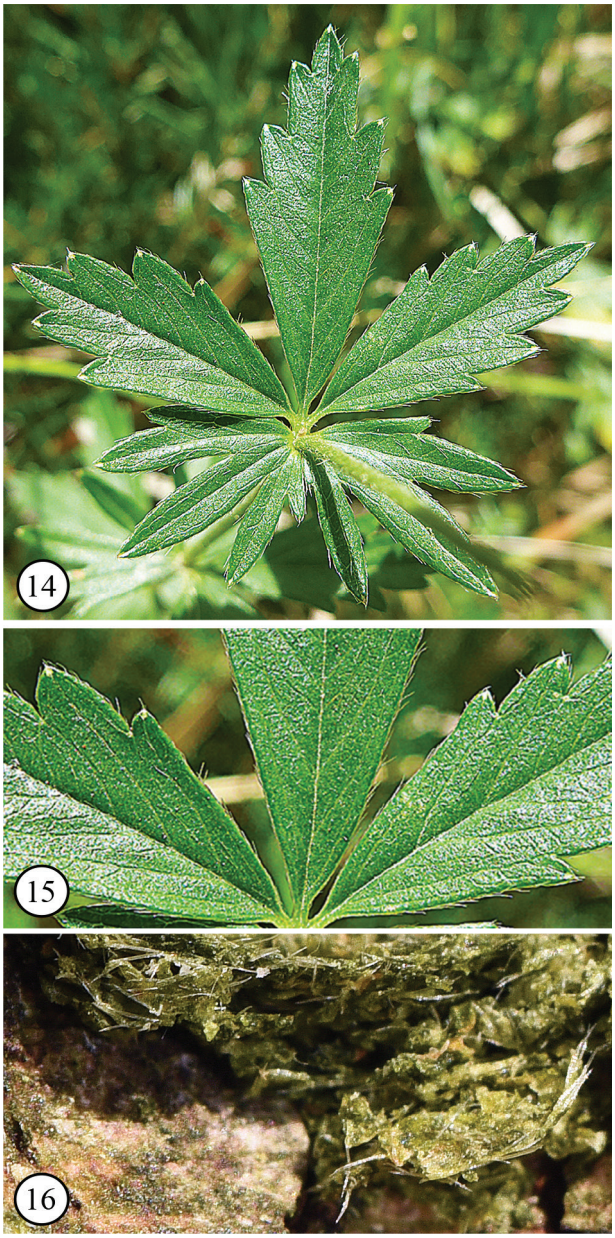
Four females of *Hoplitis robusta* were observed in the Grisons near Sedrun on 1.8.2013, near Sent on 5.7.2016 and near Curaglia on 29.6.2018 harvesting material for nest construction from green leaves of *Potentilla erecta*. DNA metabarcoding revealed that the plug of nest 2 was constructed from masticated leaves of three rosaceous herb species including *Potentilla erecta* (Tab. 3).

Table 2. Pollen composition of larval provisions in eight brood cells of *Hoplitis robusta* from two nests owned by different females; in brood cells 1–3 of nest 2, the larvae had already devoured all provisions.

		Potentilla	*Helianthemum*
nest 1	brood cell 1	90%	10%
	brood cell 2	100%	
	brood cell 3	100%	
	brood cell 4	90%	10%
	brood cell 5	100%	
	brood cell 6	100%	
nest 2	brood cell 4	100%	
	brood cell 5	100%	
total		97.5%	2.5%

Table 3. Origin of the masticated green leaves used by *Hoplitis robusta* to build the plug of nest 2 based on DNA metabarcoding.

Plant taxon	% sequence reads
Fragaria vesca (Rosaceae)	50.9
Potentilla erecta (Rosaceae)	43.6
Alchemilla spec. (Rosaceae)	5.5



Figures 14–16. 14) Leaf of *Potentilla erecta*. **15)** Close-up view of leaf of *Potentilla erecta* with indumental hairs along the leaf margins and on the leaf surface. **16)** Leaf pulp matrix of uppermost partition of the plug of nest 1 with numerous indumental hairs possibly originating from *Potentilla* and/or *Fragaria*.

Figure 17. L-shaped pupation tunnel of *Tetropium castaneum* (Cerambycidae) after emergence of the adult beetle with densely packed wood chips and fibers in the straight horizontal part of the tunnel (photo B. Wermelinger).

Although the percentage of sequence reads obtained by next-generation sequencing is only a rough indication for the relative amount of a plant taxon in the extracted DNA, the majority of the leaf material of the nest plug likely originated from *Potentilla erecta* and *Fragaria vesca*. The stereomicroscopic examination of all cell partitions and the uppermost partition of the plug of nest 1 showed that the leaf pulp matrix invariaby contained numerous white, straight and only slightly tapering hairs of about 0.5–0.8 mm length and 0.03 mm maximal width (Fig. 16). Hairs of identical shape and dimensions were found both along the margins and on the upper and lower surface of the leaves of *Potentilla erecta* and *Fragaria vesca* (Figs 14, 15). However, among the 54 plant species collected within a radius of 15 m around the nesting site, the leaves of four additional species also had such hairs, i.e. *Helianthemum* spec. (Cistaceae), *Ranunculus* spec. (Ranunculaceae), *Thymus* spec. (Lamiaceae) and *Trifolium* spec. (Fabaceae), rendering it impossible to unambiguously assign the leaf pulp hairs to a single plant taxon. Nevertheless, the presence of such hairs within the leaf pulp matrix does neither contradict the field observations described above nor the results obtained by DNA metabarcoding and suggests that the cell partitions and the plug of nest 1 might actually have been (partly) built from masticated leaves of *Potentilla* and/or *Fragaria*.

Discussion

Nesting site and nest architecture

The nest architecture of the two nests of *Hoplitis robusta* from the Swiss Alps corresponds well with that of nests found in drilled borings of artificial trap nest blocks in the Rocky Mountains/USA (Clement and Rust 1975). The six North American nests were built in borings with a diameter of 4–5 mm and contained 3–14 linearly arranged and 5.5–11 mm long brood cells; the 0.3–1 mm thin cell partitions were constructed from masticated leaves and the 10–14 mm long nest plugs consisted of 8–13 successive layers of leaf pulp. However, in contrast to the findings of the present study, three North American nests contained 3–18 mm long vestibule cells following the last brood cell and some cell partitions had small pieces of wood embedded in the leaf pulp matrix. Whether the construction of vestibule cells and the combination of leaf pulp with additional material also occurs in European populations of *H. robusta* is unknown. Interestingly, nests of *Hoplitis maritima* (Romankova) – an eastern Asian representative of the subgenus *Formicapis* – were discovered in cerambycid beetle burrows in 4–5 cm thick branches of dead fallen conifers (Romankova 1985), exactly corresponding to the nesting sites of the two females of *H. robusta* in the Swiss Alps; these nests contained 2–6 linearly arranged brood cells and leaf pulp served as material for the construction of both cell partitions and nest plug. The identical nesting biologies of *H. robusta* and *H. maritima* suggest that the use of beetle burrows in dead wood as nesting site and of masticated leaves as nest building material are subgeneric traits of the subgenus *Formicapis*.

Females of *Hoplitis robusta* possess unusually large and powerful mandibles (Fig. 2). As such mandibles do not appear to be adaptive in collecting floral resources nor in harvesting and masticating green leaves, their function remains enigmatic. We hypothesize that these powerful mandibles have evolved as a tool to clean L-shaped pupation tunnels of cerambycid beetles immediately following nest site selection. After emergence, adults of cerambycid beetle species, which gnaw L-shaped tunnels in the last larval instar for pupation, use to leave behind substantial amounts of densely packed wood chips and fibres in the straight horizontal part of the tunnel (Fig. 17). The strong female mandibles might provide the needed strength to remove the wood debris out of the tunnel. A similar cleaning function has been hypothesized for the huge female mandibles of *H. maritima* (Romankova 1985, Müller and Mauss 2016).

Pollen hosts

The composition of the female scopal loads, the pollen content of the brood cells, field observations of flower visiting females and the highly efficient pollen collecting behaviour all suggest that *Hoplitis robusta* is strongly dependent on *Potentilla* (Rosaceae) for its reproduction. Since the pollen of *Potentilla* could not reliably be distinguished from that of *Fragaria* by the method used in the present study (see Methods), it cannot be excluded that *H. robusta* occasionally also exploits flowers of *Fragaria vesca*. However, as the flowering period of *F. vesca* and the flight period of *H. robusta* at most marginally overlap, *Fragaria* is not expected to be a regular pollen host of *H. robusta* in the Alps. A preference for the pollen of herbaceous Rosaceae has also been recorded for eastern Asian *Hoplitis* species of the subgenus *Formicapis*: *H.*

maritima is oligolectic on *Fragaria* and *Potentilla* (Romankova 1985, Proshchalykin in Quest 2009), and several pollen loads of *H. excisa* (Morawitz) were composed of Rosaceae pollen, mainly of the *Potentilla* type (Müller and Mauss 2016). This suggests that the preferential or exclusive use of herbaceous Rosaceae as pollen hosts is a subgeneric trait of *Formicapis*.

The larval provision of a single brood cell of *Hoplitis robusta* from the Rocky Mountains/USA contained pollen possibly from a legume, such as *Astragalus* or *Trifolium* (Clement and Rust 1975). However, given the uncertainty in pollen identification admitted by the authors and the negligible amount of Fabaceae pollen in the larval diet of *H. robusta* in the Alps (see Tab. 1), the larval provision might possibly have consisted of pollen of the *Potentilla* type, which – albeit distinctly smaller – has a similar morphology and shape as pollen grains of *Astragalus* and *Trifolium*. This assumption is in line with flower records of *H. robusta* from North America, which originate mainly from herbaceous Rosaceae, primarily *Potentilla* (Ascher and Pickering 2018).

Nest building material

The use of green leaves of *Potentilla erecta* as nest building material by *Hoplitis robusta* is documented both by field observations and DNA metabarcoding and is also suggested by the stereomicroscopic analysis of the leaf pulp matrix, clearly indicating that *Potentilla* leaves play an important role for the construction of cell partitions and nest plugs in *H. robusta.* However, *Potentilla* was not the exclusive source, two other rosaceous herbs were also confirmed as suppliers of nest building material, i.e. *Fragaria vesca* and possibly to a lesser and only marginal degree *Alchemilla* spec. The fact that these three taxa all belong to the Rosaceae is surprising and suggests that the selection of leaves for manufacturing leaf pulp is far from accidental and that the leaves of Rosaceae species might possess particularly favourable properties as nest building material. In fact, eight Central European osmiine bee species belonging to two genera and six subgenera were recorded to harvest leaf material for nest construction from rosaceous genera, such as *Fragaria, Potentilla, Rosa* and/or *Sanguisorba*, i.e. *Hoplitis* (*Alcidamea*) *claviventris* (Thomson), *Osmia* (*Erythrosmia*) *andrenoides* Spinola, *O.* (*Helicosmia*) *aurulenta* (Panzer), *O.* (*Helicosmia*) *leaiana* (Kirby), *O.* (*Hoplosmia*) *spinulosa* (Kirby), *O.* (*Melanosmia*) *parietina* Curtis, *O.* (*Melanosmia*) *pilicornis* Smith and *O.* (*Neosmia*) *bicolor* (Schrank) (Müller 1994, 2018a and references therein, Prosi et al. 2016). Although the very poor knowledge on the plant spectra exploited by leaf masticating megachilid bees for nest construction renders any conclusions premature, it is well conceivable that some species including *H. robusta* might be specialized with respect to the plants they use for manufacturing leaf pulp. Interestingly, experiments with the North American *Osmia bruneri* Cockerell in flight cages recently revealed that leaves of *Malva neglecta* and *Sphaeralcea ambigua* (both Malvaceae) as well as of *Oenothera elata* (Onagraceae) were readily accepted by the females as leaf pulp sources, whereas those of *Callirhoe involucrata* and *Hibiscus syriacus* (Malvaceae) as well as of *Cercis canadensis* (Fabaceae) were ignored, suggesting strong preferences for the plants exploited for leaf pulp (Cane submitted).

Conclusions

Among the Central European megachild bees, *Hoplitis robusta* is an exception in that it harvests floral resources and nest building material from the very same plant taxon, i.e. *Potentilla*. Though the dependence on *Potentilla* is not complete, neither for collecting floral resources nor for harvesting nest building material, *Potentilla* plays a key role in the life history of *H. robusta*. The findings of the present study suggest that large stands of *Potentilla erecta* in close neighbourhood to sun-exposed dead wood fulfill all requirements for population survival of *H. robusta* in the subalpine zone of the Alps. Pollen of *Potentilla* is also crucial for the reproduction of other alpine bee species. *Andrena tarsata* Nylander and *Panurginus herzi* Morawitz are strictly specialized on *Potentilla*, while in several polylectic species, such as *Andrena amieti* Praz, Müller & Genoud, *Andrena coitana* (Kirby) or *Panurginus montanus* Giraud, pollen of *Potentilla* often represents a considerable proportion of the collected pollen (Westrich 1989, Müller 2018b, Praz et al. submitted). Thus, species of *Potentilla* and particularly *P. erecta* play a significant role in sustaining populations of numerous bee species in alpine habitats.

Acknowledgements

We gratefully acknowledge the help of the following colleagues who generously permitted pollen removal from *Hoplitis robusta* specimens of their collections or under their curation: F. Amiet (Solothurn), H. Baur (Naturhistorisches Museum Bern), R. Eastwood (ETH Zürich), K. Hirt (Menziken), A. Freitag (Musée de Zoologie Lausanne), F. Gusenleitner and M. Schwarz (Biologiezentrum Linz) and S. Liersch (Bündner Naturmuseum Chur). B. Wermelinger (WSL Birmensdorf) and U. Bense (Mössingen) provided information on the biology of Cerambycidae. J. Cane (Utah State University) made a still unpublished manuscript on the leaf pulp sources of *Osmia bruneri* available to us. T. Janke (Eurofins, Ebersberg) provided technical information on metabarcoding. B. Wermelinger kindly provided the photo of figure 17. Comments by Jack Neff, Stefan Dötterl and Jessica Litman improved the manuscript.

References

Amiet F, Herrmann M, Müller A, Neumeyer R (2004) Apidae 4: *Anthidium*, *Chelostoma*, *Coelioxys*, *Dioxys*, *Heriades*, *Lithurgus*, *Megachile*, *Osmia*, *Stelis*. Fauna Helvetica 9: 1–274.

Ascher JS, Pickering J (2018) Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). http://www.discoverlife.org/mp/20q?guide=Apoidea_species [Accessed 2.11.2018]

Beug HJ (2004) Leitfaden der Pollenbestimmung für Mitteleuropa and angrenzende Gebiete. Pfeil Verlag, München, 542pp.

Cane J (submitted) *Osmia bruneri* bees (Megachilidae) using fruit pulp for nest construction. Apidologie.

Clement SL, Rust RW (1975) The biology of *Hoplitis robusta* (Hymenoptera: Megachilidae). Entomological News 86: 115–120.

Ebmer AW (1997) Hymenopterologische Notizen aus Österreich – 7 (Insecta: Hymenoptera: Apoidea). Linzer biologische Beiträge 29: 45–62.

Ebmer AW (2001) Hymenopterologische Notizen aus Österreich – 14 (Insecta: Hymenoptera: Apoidea). Linzer biologische Beiträge 33: 435–460.

Ferton C (1890) Recherches sur les moeurs de quelques espèces algériennes d'hyménoptères du genre *Osmia*. Actes de la Société Linnéenne de Bordeaux 44: 201–209.

Ferton C (1892) Sur les moeurs de quelques Hyménoptères de la Provence du genre *Osmia*. Actes de la Société Linnéenne de Bordeaux 45: 231–240.

Ferton C (1894) Seconde note sur les moeurs de quelques Hyménoptères du genre *Osmia* Panzer principalement de la Provence. Actes de la Société Linnéenne de Bordeaux 47: 203–214.

Ferton C (1897) Nouvelle observations sur l'instinct des hyménoptères gastrilégides de France et de Corse. Actes de la Société Linnéenne de Bordeaux 52: 37–50.

Frey-Gessner E (1880) Exkursionen im Sommer 1879. Mittheilungen der Schweizerischen Entomologischen Gesellschaft 5: 515–589.

Frey–Gessner E (1908–1912) Hymenoptera, Apidae. Fauna insectorum Helvetiae. Vol. II. Körber, Schaffhausen, 319pp.

Gotlieb A, Pisanty G, Rozen JG, Müller A, Röder G, Sedivy C, Praz C (2014) Nests, floral preferences, and immatures of the bee *Haetosmia vechti* (Hymenoptera: Megachilidae: Osmiini). American Museum Novitates 3808: 1–20. https://doi.org/10.1206/3808.1

Kopf T (2008) Die Bienenfauna (Hymenoptera: Apidae) des Schlerngebietes (Südtirol, Italien) mit Angaben zu den Artengemeinschaften ausgewählter Lebensräume. Gredleriana 8: 429–466.

Müller A (1994) Die Bionomie der in leeren Schneckengehäusen nistenden Biene *Osmia spinulosa* (Kirby 1802) (Hymenoptera, Megachilidae). Veröffentlichungen für Naturschutz und Landschaftspflege Baden-Württemberg 68(69): 291–334.

Müller A (2018a) Palaearctic Osmiine Bees. ETH Zürich. http://blogs.ethz.ch/osmiini [Accessed 13.9.2018]

Müller A (2018b) Pollen host selection by predominantly alpine bee species of the genera *Andrena*, *Panurginus*, *Dufourea*, *Megachile*, *Hoplitis* and *Osmia* (Hymenoptera, Apoidea). Alpine Entomology 2: 101–113.

Müller A, Kuhlmann M (2008) Pollen hosts of western palaearctic bees of the genus *Colletes* (Colletidae) – the Asteraceae paradox. Biological Journal of the Linnean Society 95: 719–733. https://doi.org/10.1111/j.1095-8312.2008.01113.x

Müller A, Mauss V (2016) Palaearctic *Hoplitis* bees of the subgenera *Formicapis* and *Tkalcua* (Megachilidae, Osmiini): biology, taxonomy and key to species. Zootaxa 4127: 105–120. https://doi.org/10.11646/zootaxa.4127.1.5

Praz C, Müller A, Genoud D (submitted) Hidden diversity in European bees: *Andrena amieti* sp. n., a new alpine bee species related to *Andrena bicolor* (Fabricius, 1775) (Hymenoptera, Apoidea, Andrenidae). Alpine Entomology.

Prosi R, Wiesbauer H, Müller A (2016) Distribution, biology and habitat of the rare European osmiine bee species *Osmia* (*Melanosmia*) *pilicornis* (Hymenoptera, Megachilidae, Osmiini). Journal of Hymenoptera Research 52: 1–36. https://doi.org/10.3897/jhr.52.10441

Quest M (2009) Artbestand, Ökologie und Habitatwahl von Bienen ausgewählter Offenlebensräume im Lazovski Zapovednik (Ferner Osten Russland). Entomofauna, Supplement 15: 1–357.

Romankova TG (1985) A new subspecies of *Formicapis robusta* (Hym. Megachilidae) from Primorye area. Vestnik Zoologii 6: 66–68.

Rozen JG, Özbek H, Ascher JS, Sedivy C, Praz C, Monfared A, Müller A (2010) Nests, petal usage, floral preferences, and immatures of *Osmia (Ozbekosmia) avosetta* (Megachilidae: Megachilinae: Osmiini), including biological comparisons with other osmiine bees. American Museum Novitates 3680: 1–22. https://doi.org/10.1206/701.1

Schwarz M, Gusenleitner F, Westrich P, Dathe HH (1996) Katalog der Bienen Österreichs, Deutschlands und der Schweiz (Hymenoptera, Apidae). Entomofauna, Supplement 8: 1–398.

SwissBeeTeam (2018) Online Atlas of the Swiss Wild Bees. InfoFauna, Neuchâtel. http://swisswildbees.ch [Accessed 13.9.2018]

Westrich P (1989) Die Wildbienen Baden-Württembergs. Ulmer, Stuttgart, 972 pp.

Supplementary material 1

Computed tomography (CT) movie of nest 1 of *Hoplitis robusta*

Authors: Andreas Müller, Henning Richter

Data type: species data

Explanation note: Computed tomography (CT) movie of nest 1 of *Hoplitis robusta*; note the vertical nest plug consisting of several successive layers of leaf pulp and the six brood cells in the horizontal part of the L-shaped cerambycid tunnel.

Link: https://doi.org/10.3897/alpento.2.30158.suppl1

Supplementary material 2

Computed tomography (CT) movie of nest 2 of *Hoplitis robusta*

Authors: Andreas Müller, Henning Richter

Data type: species data

Explanation note: Computed tomography (CT) movie of nest 2 of *Hoplitis robusta*; note the vertical plug consisting of several successive layers of leaf pulp and the five brood cells in the horizontal part of the L-shaped cerambycid tunnel.

Link: https://doi.org/10.3897/alpento.2.30158.suppl2